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MALDI/Mass Spectrometry of "Normal Appearing" and Dystrophic Axons in Spinal Cord of Multiple Sclerosis (MS)

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14. ABSTRACT
Progressive myelopathy is the predominant feature of secondary and primary progressive MS. It is likely responsible for much of the disability seen in patients with MS. We postulated that the unremitting nature of the disorder is not likely to be due to loss of myelin, but rather a disorder of axons of the spinal cord. We therefore examined the protein profile of regions of normal appearing white matter between MS patients and controls using MALDI/spectrometry. Our preliminary studies show a difference in the signature of proteins in areas of normal appearing white matter between MS and controls. Our next step is to identify the nature of the proteins which are different between MS and controls.

15. SUBJECT TERMS
Mutliple sclerosis; demyelination; MALDI/spectrometry; myelopathy

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Table of Contents

Annual SummaryReport

Introduction: Although considered to be a prototypic inflammatory demyelinating disease, there is incontrovertible evidence that destruction of axons is a crucial feature of the disease and most likely responsible for progression of disability [1, 2] . In addition, there is evidence to suggest that axonal loss occurs early and is widespread in the white and grey matter [3]. There is recent awareness that the amount of demyelination (volume and number of lesions seen on MRI) have little or no correlation with disease progression and is poorly correlated with disability [4]. Progression in MS is characterized by clinical findings consistent with corticospinal (CS) tract dysfunction that is usually bilateral but asymmetrical [5]. Pathology of axons in the corticospinal tracts shows decrease in axonal density and size. There appears to be preferential loss of small axons and relative preservation of large diameter axons, in areas of spinal cord that do not show demyelination [4]. The cause of axonal loss and the protein changes in the small dystrophic axons when compared to the "normal appearing" large diameter axons are unknown.

Using MALDI/mass spectrometry we will determine:
(a) if "normal appearing" large diameter axons in CS tracts of MS patients differ from those seen in controls.
(b) the protein profile of dystrophic small axons in CS tracts differ when compared with normal large axons and those of non MS controls.

Body: We obtained spinal cord tissue from brain and spinal cord banks in the U.S. and the Netherlands. The tissue obtained from the U.S. was not found suitable and, therefore, we concentrated our efforts from the bank in the Netherlands. Regions of the cervical spinal cord were stained with hemotoxylin-eosin and with Luxol fast blue (LFB) and areas of demyelination and inflammation assessed. Using antibodies to neuro-filament the large and small axons on the anterior, lateral and posterior columns of the spinal cord were identified.

We identified the following regions from the spinal cord for MALD/spectrometric studies: a) normal appearing white matter with large axons in MS and controls, b) normal appearing white matter with small caliber axons from MS and healthy controls, c) demyelinated regions of spinal cord.

These regions were micro dissected and subjected to MALDI and the results are currently being analyzed.

Key Research accomplishments: Defining by MALDI regions of normal appearing white matter between MS and controls which differ in their MALDI signature.

Reportable outcomes: None

Conclusions: We had asked for a no cost extension of our project because of technical issues which delayed our experiments. Firstly, the tissue which we obtained from the U.S. was not sufficiently good quality and we had to obtain more material from the brain bank in the Netherlands for our study. This delayed our start of the experiments. Between the months of April to June of 2012, there was a major failure of the MALD/spectrometric instrument and, therefore, our samples could not be analyzed in time. We are, however, on target to analyze the parent proteins which differ between MS patients and controls in areas of normal appearing white matter, which show normal caliber of large and small axons.

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Abbreviations:
MALDI = Matrix-assisted laser desorption ionization
CS= corticospinal
MS= Multiple sclerosis
MRI= Magnetic resonance imaging
LFB= Luxol fast blue